REMARKS

Claims 1, 2, 4, 6-8, 9. 11, 15 and 16 are all the claims pending in the application. Claim 10 has been canceled. No new matter has been introduced and entry of the amendment and reconsideration are respectfully requested.

Applicants thank to the Examiner that some of previous rejections have been withdrawn.

I. Rejection of Claims 10 and 16 under 35 U.S.C. 112, 1st paragraph

Claim 10 stands rejected under 35 U.S.C. § 112, firs paragraph, as failing to comply with the written description requirement and the enablement requirement. Claim 10 has been canceled thereby rendering the rejections of Claim 10 moot.

Claim 16 has been rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. Applicants respectfully traverse the rejection.

The Office Action asserts that the construction of plasmid pAC104CysK requires the plasmid p10499A and the construction of the plasmid pED1L-12p40 requires the leptin expression vector of Jeong and Lee (Appl. Environ. Microbiol., 65:3027-32, 1999), neither of which is fully disclosed in the specification or shown to publicly available.

Applicants respectfully submit that the specification describes that the plasmid pAC104CysK was constructed according to the teachings of Park et al., *FEMS. Microbiol. Lett.* 214:217, 2002 (page 218, right column). See Park et al. is referenced in the specification of the present application (page 8, lines 17-18) and the construction of the plasmid pAC104CysK is fully disclosed in the specification, at page 8, lines 15-20 to enable the one skilled in the art to

make and /or use the invention. Park et al. was published prior to the invention of the instant application and a copy thereof was submitted in the IDS filed February 27, 2004. Park et al describes commercial sources of the materials used for the construction of the plasmid pAC104CysK. For example, it teaches that pTrc99A was obtained/available from Pharmacia Biotech, Uppsala, Sweden.

The specification of the present application describes that the leptin expression vector, pEDOb5, which was employed in the preparation of plasmid pEDIL-12p40, was prepared by the description of Jeong et al., *Appl. Environ. Microbiol.* 65:3027, 1999 ("Jeong (1999)"). Table 1 and Fig. 1 on page 3028 of Jeong (1999) describe the restriction map and the construction of the vector pEDOb5. Also, the description on the right column on page 3028 explains how to make and use the vector pEDOb5. The materials used to construct the vector pEDOb5, for example, pET21c has been available from Novagen. See Table 1 of Jeong (1999). The plasmid pUCOb was constructed by subcloning a certain gene into pUC19 at the *Eco*RI site. Section "Materials and Methods" of Jeong (1999), on page 3027, right column.

In addition, the specification describes that the preparation of hG-CSF expression vector (pEDCSFMll) is described in Jeong et al., *Protein Expr. Purif.* 23:311–318, 2001 ("Jeong (2001)"). In particular, the section "Materials and Method" and Table 1 of Jeong (2001) explain the sources of materials used for the construction of pEDCSFM11. Also, Fig. 1 and Fig. 3 show the schematic diagrams and restriction maps of pEDCSFm11, and the section "Results" explains in detail how to make and use each material to construct pEDCSFM11.

Jeong (1999) and Jeong (2001) are referenced in the specification of the instant application and copies thereof were submitted in the IDS filed February 27, 2004.

Therefore, Applicants respectfully submit that Claim 10 complies with the enablement requirement and request that the rejection be withdrawn.

II. Rejection of Claims 1, 2, 4, 6-9, 11 and 15 under 35 U.S.C. 103(a)

Claims 1, 2, 4 6-9, 11 and 15 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Ramirez et al. and Lamouse-Smith et al. in view of martens et al., Swiss-Prot Accession No. P29460, Hatamoto et al. (JP 09/009982) and Koonin et al.

Claims 1 and 2 have been amended to change the term "cysteine synthase (CysK) gene" to recite "cysK."

Ramirez teaches that recombinant protein production in *E. coli* can be improved by increasing available levels of amino acids present in the recombinant protein rather than the levels of amino acids. Also, Swiss-Prot accession No. P29460 and Koonin suggest that IL-12 p40 has as much as 3 times more cysteine than the average *E. coli* protein. However, Lamouse teaches that, when an amino acid is present in a recombinant protein at levels significantly higher than that present in host cellular proteins, the amino acid becomes a limiting factor in expression level. In other words, an increase in specific amino acid level does not directly induce an increase in specific protein production; rather, the production of specific amino acid is inhibited by feedback inhibition regulatory network.

Also, amino acid production (metabolites) is different from foreign protein production system. Protein production comprises more complicated steps than that of amino acid

production, so, enzymes, precursors etc. involved in protein synthesis should be well balanced. If cysteine increases in host cellular protein, cellular regulating system will inhibit cysteine production and IL-12 p40 serine-rich protein production will not increase.

As shown in Example 4 of the present invention, when the leptin expression plasmid was expressed alone, the expression of leptin reached the maximum after 8 hours of the induction. By contrary, when the leptin expression plasmid was coexpressed along with the plasmid pAC104CysK, the expression of leptin reached the maximum after 2 hours of the induction. This indicates that metabolism was inhibited since *E. coli* was cultured at high concentration.

The present invention solves this problem and a greater amount of serine-rich protein was produced for a shorter time to reduce initial metabolic burden. As described in Example 1, the present inventors verified that all amino acid syntheses were inhibited in the culture solution of high concentration, and thus, serine family amino acid synthesis pathway was substantially inhibited by leptin overexpression. Therefore, to reduce synthetic metabolism of serine family amino acids (CysK, GlyA), cysK gene is employed, according to the present invention. None of the cited references teach or suggest a process or vectors which render the maximum productivity of serine-rich protein for a short time, as recited in the present claims.

As described in Example 4, the leptin production time was unexpectedly reduced from 8 hours to 2 hours.

Also, it is reported that serine-rich protein production in *E. coli* is difficult (Bula C et al., *Protein Expr. Purif.*, 7:92, 1996). There have bee two solutions well-known in the art: i)

blocking a pathway for feedback inhibition of cysteine (using cysE variants, as taught in Hamamoto); and ii) removing a degradation pathway of produced cysteine.

cysE gene is one of major factors in the production of cysteine, and the amount of cysteine in cells is auto-regulated by the feedback inhibition. When the amount of cysteine is excessive, cysteine is degraded by the degradation pathway. Since cysE gene is also auto-regulated, Hamamoto uses a cysE variant, Met256-point-mutated-Ile256 to prevent the feedback inhibition, in order to produce a large amount of cysteine without feedback inhibition. In other words, Hamamoto just teaches an amino acid synthesis and does not suggest or teach the currently claimed invention.

Hamamoto teaches a control of cysK and cysE using a lac promoter (inducible promoter). By contrary, the present invention uses p104 promoter (constitutive promoter). Generally, an inducible promoter is used to produce a target protein in large quantities for a short period of time. In addition, the production of a specific protein is determined by the rate at which the specific protein is produced depending on the amount of whole protein using SDS-PAGE. An inducible promoter, which is a strong promoter, may be used when an increase in the cysK expression is desired. The use of inducible promoter, however, results in a relatively decreased production rate of a specific protein due to the increased proportion of the produced cysK protein, based on the total amounts of proteins. Therefore, Hamamoto teaches a cysteine production rather than the activation of serine-rich protein synthetic pathway.

In contrast, the present inventors used a constitutive promoter for the activation pathway which is related to cysK and thus cysK is expressed in an amount at which the feedback inhibition by cysteine overexpression can be avoided.

In the case of recombinant bacteria according to present invention, cysK is expressed before the initiation of serine-rich protein synthesis pathway and, thus, the cysK gene expression pre-activates the serine-rich protein synthesis pathway. By contrary, in conventional methods described by Hamamoto, the serine-rich protein synthesis pathway is initiated by a gene encoding serine-rich protein, followed by the expression of cysK.

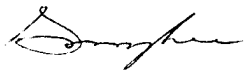
According to the present invention, even when a serine-rich protein such as leptin is overexpressed, the production of the serine-rich protein is not inhibited by feedback inhibition, and the pathway maintains the protein production using an intermediate (O-acetyl intermediate) and other metabolites which are produced previously by serine-rich synthesis pathway activated by cysK expression. As discussed above, the time period for the maximum production of the protein in the convention bacteria and that of the inventive bacteria were 8 hours and 2 hours, respectively, showing an unexpected advantage of the present invention. In an experiment employing cysE gene, instead of cysK gene, it was found that cysE gene amplification did not induce an increase of serine-rich proteins.

For the reasons discussed above, it is respectfully submitted that the rejection is not sustainable and applicants request that the rejection be withdrawn.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



Sunhee Lee
Registration No. 53,892

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860

WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: December 26, 2006